A Multivariate Analysis of Factors Affecting Mercury

Availability in Dragonfly Nymphs (Order: Odonata)

Inhabiting Wetland Ecosystems

C. M. Headon

Department of Zoology

University of Toronto

Toronto, Ontario

and

Ronald J. Hall

Dorset Environmental Centre

Box 39

Dorset, Ontario [1]

January 1997

1. Email: ronjhall@sympatico.ca

Abstract

Fifteen wetlands were examined to determine the influence of sediment and water chemical parameters on mercury bioavailability in dragonfly nymphs (Order: Odonata; suborder: Anisoptera;   Genus: *Cordulia*). Wetland types included mineral poor fens, circumneutral minerotrophic fens and marshes. Mercury concentrations in dragonfly nymphs ranged from 0.041 to 0.402 ug.g^{-1} dry mass and were significantly ($p < 0.001$) different among the 15 wetlands. Multivariate statistical procedures showed that sediment total sulfur (S) concentration explained the greatest amount of the among wetland variation in Hg concentrations in anisopteran numphs. With increasing concentrations of total S in the sediments there was a decrease in nymph Hg concentrations. Surface water chemistry appeared not to have a direct affect on Hg bioavailability.

Key words: wetlands, mercury, dragonfly, *Cordulia*, Odonata, sediment and water chemistry, Ontario

Introduction

Many lakes in Ontario remote from anthropogenic sources of mercury (Hg) contain predatory fish with Hg levels exceeding the Canadian $0.5 \ \mu g.g^{-1}$ wet weight upper limit for safe consumption (OMOEE and OMNR 1993). Atmospheric deposition of Hg is believed to be a significant source of Hg to these systems (Evans 1986; Mierle 1990). However, the total concentration of a metal in the water and/or sediment does not provide a good indication of metal availability for uptake by aquatic biota (Luoma 1989; Hare 1992). The bioavailability of a metal is controlled by its speciation in the water and sediment, which is a function of a number of chemical and biological processes (Luoma 1989). In natural aquatic systems, a relatively large number of chemical variables have been indicated as being important factors affecting Hg bioavailability. For example, in relatively pristine areas, water chemical parameters representative of lake acidity (pH and alkalinity) (Wren and MacCrimmon 1983; McMurtry et al. 1989; Cope et al. 1990; Suns and Hitchin 1990; Wren et al. 1991), dystrophy (dissolved organic carbon, total organic carbon, colour and Fe) (McMurtry et al. 1989; Swain and Helwig 1989; Grieb et al. 1990; Wren et al. 1991; Fjeld and Rognerud 1993) and hardness (Ca, Mg and conductivity) (Wren and MacCrimmon 1983; McMurtry et al. 1989; Wren et al. 1991) have all been indicated as being significantly correlated with fish Hg concentrations. Mercury concentrations in crayfish have also been shown to be significantly correlated with lake acidity, hardness and dystrophy (Allard and Stokes 1989; Headon 1994).

Wetlands are unique systems that differ substantially from lake ecosystems. Even in relatively well buffered landscapes, wetlands often have lower alkalinities, substantially higher DOC levels and greater acidity than lakes that occupy the same catchment. These parameters have been implicated in explaining high Hg levels in fish (e.g. McMurtry et al. 1989; Wren et al. 1991). Furthermore, wetland sediments often have a much higher organic content than lake sediments, a factor which has been demonstrated to be a significant negative correlate of Hg concentrations in benthic biota (Breteler et al. 1981; Langston 1982). It is uncertain how sediment characteristics and water chemistry of different wetland systems will interact to influence Hg bioavailability. The interactions among various physicochemical factors, with regards to their role in moderating Hg availability in aquatic systems, is very complex. This has resulted in a number of conflicting findings. For example, while DOC has been implicated as a positive correlate of Hg levels in fish inhabiting drainage lakes, high DOC has been shown to be negatively correlated

with fish Hg concentrations in seepage lake systems (no inlet or outlets) (Grieb et al. 1990).

We know very little about the role of wetland physicochemistry in modifying biological availability and accumulation of Hg in biota (invertebrates, fish and wildlife), whether those biota occur in the wetland environment itself or aquatic systems with hydrologic ties to wetlands. Our lack of knowledge is somewhat surprising considering that 14% of Canada is covered with wetlands (National Wetlands Working Group 1988). In contrast, only 8% of Canada is covered by lakes (Roulet 1990). However, in the past, the importance of potential Hg contamination of sport fish has focused much of the research on lake environments. Nevertheless, recent studies have indicated that wetlands may be a significant source of Hg to lakes remote from known point sources of Hg (Lee and Hultberg 1990; Mierle and Ingram 1991; St. Louis et al. 1994). St. Louis et al. (1994) recently demonstrated that wetlands are an important source of CH_3Hg to boreal forest ecosystems. Lee and Hultberg (1990) concluded that the concentration of CH_3Hg in catchment run-off and lake water were related to the area of wetland coverage in the catchment. Important processes may be occurring within wetlands that result in increased Hg availability to biota as well as increased loading to the rest of the watershed.

This paper examines the influence of sediment and water chemical parameters on Hg bioavailability in wetlands through the use of principal components analysis (PCA) and stepwise multiple linear regression. For the purposes of this study, Hg bioavailability has been defined by total Hg concentrations in dragonfly nymphs. The results of this study will provide background information for more detailed mechanistic studies of factors affecting Hg bioavailability in wetland environments.

Methods

Location and Description of Study Wetlands

Pool water samples, sediment cores and dragonfly nymphs were collected from each of 15 wetlands located in the Muskoka-Haliburton region of south-central Ontario, and each of 11 wetlands located in North Bay, Ontario. Each wetland was sampled only once during the months of July and August of 1993. All wetlands were remote from known point sources of Hg. Dragonfly nymphs have been analyzed for total Hg in sufficient numbers from 15 of the 26 study wetlands; three of which are located in North Bay (wetlands N, number 7 and H). Only data from this 15 wetland subset will be presented in this paper. The study wetlands included a variety of wetland types (mineral poor fens, circumneutral minerotrophic fens and marshes) representing a gradient in alkalinity, pH, water column DOC concentrations and sediment organic content.

Sample Collections and Chemical Determinations

Wetland pool water samples were collected for a number of chemical parameters. Samples were taken at a depth of approximately 30 cm below surface at the centre of each wetland. Water samples were submitted to the Dorset Research Centre, Ontario Ministry of the Environment and Energy (OMOEE), for chemical analyses. Details of the analytical procedures utilized for the water analyses are described in Outlines of Analytical Methods (OMOE 1981). Duplicate sediment/peat samples (upper 2.5 cm) were collected with a modified gravity corer from each odonate collection site. Organic content (measured as percent loss on ignition), total sulphur, and non-residual iron and manganese were determined by OMOEE.

Immediately following the collection of sediment/peat samples, Anisoptera (dragonfly) nymphs (Order: Odonata) were collected from each sampling site with an aquatic D-net and plastic sieve. Anisoptera were chosen as the indicator organism since they are known to inhabit aquatic systems with a diverse range in pH (Pollard and Berrill 1992) and are common in wetland environments (Hilton 1987). Only Hg data for dragonfly nymphs of the genus *Cordulia* will be presented in this paper.

In the field, nymphs were placed five to a polyethylene container filled with wetland water. Containers were placed in coolers and brought back to the laboratory were they were held in the

dark at 19°C for 48 h to allow for gut clearance. Nymphs were then rinsed with distilled deionized water, placed in acid washed glass scintillation vials, covered with parafilm and frozen for later analysis.

The procedure followed for the determination of total Hg levels was based on that outlined by Rasmussen et al. (1981). Nymphs were first thawed and total length was measured. They were then identified to genus by utilizing the keys of Walker (1958), and Walker and Corbet (1975). Odonates were rinsed in distilled deionized water, blotted dry to remove excess water and weighed on a top-loading electronic balance. Following this, nymphs were dried to constant weight at 60°C (approximately 48 h) and then weighed to the nearest 0.1 mg. Individuals were placed in borosilicate glass test tubes (one nymph per tube) and 2.0 mL of a 4:1 mixture of H_2SO_4 and HNO_3 was added. Glass marbles were placed over tube openings and the sample test tubes were heated on an aluminum hot block at 250°C for 8 to 10 h. Cooled samples were diluted with 8.0 mL of distilled deionized water. Total Hg was determined for each sample by cold vapour atomic absorption spectroscopy.

All Hg determinations are based on and reported as dry weights. Conversion of dry weight concentrations of Hg to wet weights can be obtained by multiplying by 0.17. This conversion factor is the mean of the dry weight to wet weight ratios for 130 individuals (SD = 0.03).

A run consisted of five procedural blanks, three to five samples of NIST (U.S. National Institute of Standards and Technology) oyster No. 1566a and a 10 ng Hg spike to reagent blank in triplicate. Between-run and mean within-run precision (n = 11 runs) for oyster tissue were 7.0% and 4.6 ± 2.2% (± SD), respectively. Precision is expressed as the relative standard deviation (RSD) (standard deviation as a percentage of observed mean). The mean observed Hg concentration of 0.062 $\mu g.g^{-1}$ for oyster tissue was close to its certified value of 0.064 $\mu g.g^{-1}$. Mercury spikes had a mean recovery of 97%. Between-run and mean within-run precision for triplicates of Hg spikes were 3.7% and 3.5 ± 1.7% (± SD).

Data Analysis

All statistical analyses were conducted using the statistical package, SYSTAT for Windows, version 5 (Wilkinson 1992). Both whole-body Hg burdens and concentrations for *Cordulia sp.* were compared among the 15 study wetlands by using the analysis of covariance (ANCOVA) and the analysis of variance (ANOVA) statistical procedures. Residuals produced with

these procedures were examined for normality and homogeneity. Based on this examination, it was determined that in order to satisfy the requirements of the statistical tests, Hg concentrations and burdens had to be $\log_{10}$-transformed. Dry mass was $\log_{10}$-transformed to improve linearity between the covariate and the independent variable.

An analysis of covariance (ANCOVA) with dry mass as the covariate and wetland as the treatment was performed to test whether Hg levels (burdens and concentrations) were dependent on nymph mass. A test for homogeneity of slopes was first made. For both the analysis conducted on the concentration data and that on the burden data, there was no significant interaction between the covariate and the treatment ($p = 0.8$). Therefore, homogeneity of slopes was assumed and an ANCOVA model without an interaction term was fitted to the data. When dry mass did not explain a significant amount of variation in Hg levels ($p > 0.05$), a regression on mass was not required and an ANOVA was then applied with wetland as the factor. Pairwise multiple comparisons were made with the Tukey HSD test if Hg levels differed significantly amongst the wetlands ($p < 0.05$). If dry mass was indicated as being a significant predictor of Hg levels, size-corrected mean Hg levels were compared amongst the wetlands by conducting the Tukey HSD test on the estimated ANCOVA model.

As a preliminary analysis of the relationships between the environmental chemical data and the Hg levels in dragonfly nymphs, Pearson product-moment correlation coefficients were calculated between least squares $\log_{10}$ mean Hg concentrations, as well as size-adjusted least squares $\log_{10}$ mean burdens, and 19 chemical parameters. Variables were transformed, if necessary, to meet the requirements of homogeneity of variance, normality and linearity.

Sediment chemistry and water chemistry variables were analyzed separately, since the number of observations were small compared to the total number of variables. In addition, I wanted to determine the relative influence of sediment chemistry and water chemistry on Hg availability in wetlands. For each of the sediment and water chemistry data sets, the data were reduced by performing a principle component analysis (PCA) on a correlation matrix of variables in that set. Prior to performing each PCA, variables were transformed in order to meet the requirements of the analysis. Bivariate plots were constructed for all possible variable pairs and transformations were made, when necessary, in order to improve linearity and homogeneity of variance. In addition, normal probability plots (observed values of a variable versus expected values from a normal distribution) were constructed for each variable to check for normality.

Following each PCA analysis, a backwards stepwise multiple linear regression was

performed with least squares $\log_{10}$ mean Hg concentrations in *Cordulia sp.* as the dependent variable and component scores produced for each wetland on each principal component as the independent variables. Alpha-to-enter and alpha-to-remove values were set at 0.15. These values are appropriate when predictor variables are fairly independent (Wilkinson 1992). Since principal components obtained in a PCA analysis are uncorrelated linear combinations of the original variables (Manly 1986), the often encountered problem of intercorrelation amongst independent variables included in a stepwise multiple regression is avoided.

Results

A total of 130 *Cordulia sp.* from 15 wetlands were analyzed for Hg. Whole-body total Hg concentrations and dry masses for these nymphs are summarized in Table 1. Arithmetic mean Hg concentrations ranged from 0.041 to 0.402 $\mu g.g^{-1}$ dry mass. An ANCOVA test revealed that dry mass was not a significant predictor of Hg concentrations in dragonfly nymphs ($p = 0.86$). An ANCOVA test was also conducted with nymph total length ($\log_{10}$-transformed) as the covariate. As with mass, length was not a significant predictor of nymph whole-body Hg concentrations ($p = 0.26$). When an ANOVA was performed on the Hg concentration data, Hg concentrations were found to be significantly different among the 15 wetlands ($p < 0.001$). In contrast to what was concluded for Hg concentrations, nymph dry mass was a significant predictor of Hg burdens ($p < 0.001$). Mercury burdens adjusted for size were significantly different among the 15 wetlands ($p < 0.001$). Identical results were obtained when the Tukey HSD test was performed on the Hg concentration and the Hg burden data. Since a number of overlapping similarities in Hg concentrations and size-corrected Hg burdens were revealed with the Tukey HSD test, definite conclusions cannot be made. In general, Arrowhead, Deer, Illegal, Simp, N and Ideal had significantly higher Hg concentrations and size-corrected Hg burdens than Upper Dawson, Buck, Bore, Ike and Wolfhowl. Only results of statistical analyses using Hg concentrations will be reported from this point on since identical conclusions were made when statistical tests were conducted with Hg concentrations and size-corrected burdens.

Due to the large number of water chemical parameters for which data existed compared to the number of wetlands sampled, a few water chemical parameters had to be removed from the data set (Ca, Mg, total P, K, Na and Cl). Calcium and Mg were removed since they were both highly correlated with conductivity. During a preliminary analysis of the water chemistry data

with a PCA, total P and K were found to load highly onto principal components that did not explain a significant amount of the variation in the data set. Therefore, these two variables were excluded. The final two variables removed were Na and Cl. Unusually high values for these two parameters were obtained for 3 of the 15 wetlands. It was suspected that these high levels were the result of road salt input during the winter months since these wetlands were relatively close to secondary roads. Means and ranges for the 15 water chemistry variables and 4 sediment chemistry variables retained for statistical analysis are provided in Table 2. Data was available from each wetland for each variable listed.

Sediment total S and organic content (measured as percent loss on ignition - %LOI) were both significantly negatively correlated with whole-body nymph Hg concentrations at $p < 0.05$ (Table 3). However, only the correlation coefficient obtained for total S and Hg concentration was significant at $p < 0.01$ (adjusted p required for $\alpha = 0.05$). Loadings of the four sediment chemical parameters onto each of the first three principal components obtained from a PCA performed on a correlation matrix of sediment chemistry are provided in Table 4. The first three components accounted for 98% of the total variance in the data set for the 15 wetlands. Organic content had a strong negative influence on the positioning of wetlands along component one. Iron had a somewhat weaker positive influence. Component two essentially represented increasing S, Mn and Fe, and component three represented decreasing Mn. Only the first two components had eigenvalues greater than one (1.72 and 1.44). Therefore, each of the first two components appears to summarize more information than an original variable. However, based on the Broken Stick method outlined in Legendre and Legendre (1983), the first component does not explain any more variation than would be expected by chance.

Groupings of wetlands that correspond to Hg bioavailability, as measured by Hg levels in *Cordulia*, were not apparent in a plot of wetland scores along each of the first two principal components, which between them accounted for 79% of the among wetland variation in sediment chemistry (Fig. 1). Of the North Bay wetlands, only wetland N appeared to be somewhat different than the Muskoka-Haliburton wetlands with respect to sediment chemistry (Fig. 1). This wetland had relatively low Fe and Mn concentrations in the sediment. The sediment chemistry of Wolfhowl (WH) was also quite different from the other study wetlands. Iron and sulphur concentrations were relatively high.

Pearson product-moment correlation coefficients were calculated between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and the wetland scores on each of the first

three principal components. The correlation coefficients for mean Hg concentrations and component one, two and three, were 0.562, -0.514 and -0.279, respectively. The correlations coefficients calculated for component one and two were significant at $p \leq 0.05$. However, to maintain an overall significance of $p < 0.05$, the Bonferrroni adjusted correlation coefficient is $|r| > 0.592$. When a backwards stepwise multiple linear regression was run with wetland scores on each of the first three principal components as the predictor variables and $\log_{10}$ mean Hg concentrations as the dependent variable, all three components were retained (Table 5). The regression model accounted for 66% of the among wetland variation in Hg concentrations.

To assist in the interpretation of the results acquired from the PCA of the sediment chemistry, the structure of the components was simplified by performing an orthogonal varimax rotation of the first two principal components. The first two components were rotated since they both had eigenvalues greater than one and accounted for 79% of the among wetland variation in the sediment chemistry. Components three and four accounted for negligible amounts of variation. When an orthogonal rotation is utilized, the rotated components remain uncorrelated (Manly 1986). The benefit of rotating components is that mainly large and small loadings are obtained for variables on each component (Manly 1986). This improves component interpretability.

Loadings of sediment variables onto each of the two rotated principal components are given in Table 6. Total S and %LOI had high positive loadings onto PC1, and Fe and Mn had high positive loadings onto PC2. When Pearson correlation coefficients were calculated between Hg concentrations and wetland scores on each of the two rotated principal components, only PC1 was significantly correlated with Hg concentrations (Table 6). As was expected based on the Pearson correlations, backwards stepwise multiple linear regression, performed with wetland scores on each of the two rotated principal components as the predictor variables and $\log_{10}$ mean Hg concentrations as the dependent variable, retained only one predictor variable (PC1). Principal component one explained 58% of the among wetland variation in nymph Hg concentrations (Table 7). The negative relationship between Hg concentrations in *Cordulia* and wetland scores on rotated PC1 is presented in Fig. 2.

With respect to the two variables with high loadings on rotated PC1 (Table 6), %LOI accounted for 36% of the among wetland variation in dragonfly nymph Hg concentrations ($p = 0.018$). Whereas arcsine S explained 61% of the variation ($p = 0.0006$), approximately the same amount of variance accounted for by rotated PC1 (58%). The negative relationship between

nymph Hg concentrations and sediment total S is shown in Fig. 3.

None of the Pearson correlation coefficients, as well as the Spearman rank correlation coefficients, between nymph Hg concentrations and each of 15 chemical water parameters listed in Table 2 were significant at $p < 0.05$ (Table 8). Of the 15 water chemical variables, square root Al and colour were the most highly correlated with $\log_{10}$ Hg concentrations (r = 0.459 and 0.344, respectively). A PCA was performed on a correlation matrix of water chemistry for the 15 study wetlands. The first four components accounted for 87% of the total among wetland variation in water chemistry. Nine variables had loadings with an absolute value greater than 0.5 on PC1 (Table 9). Alkalinity, conductivity and DIC were strongly positively influencing the positioning of wetlands along PC2, while pH was strongly negatively influencing the positionings. Principal component three essentially represented increasing levels of nitrates, and PC4 represented increasing dissolved oxygen and F. The first four components had eigenvalues greater than 1 (5.95, 4.23, 1.56 and 1.34). Therefore, each of the first four components appears to summarize more information than an original variable. However, based on the Broken Stick method outlined in Legendre and Legendre (1983), only the first two components explain more variation in the data set than would be expected by chance.

Groupings of wetlands that correspond to Hg bioavailability were not apparent from a plot of wetland scores along PC1 and PC2, which between them accounted for 68% of the total among wetland variation in water chemistry (Fig. 4). The North Bay wetland H was quite different from the other wetlands in terms of water chemistry (Fig. 4). This wetland had relatively high alkalinity and conductivity values. Pearson correlation coefficients between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and the scores on each of the first four components were *not* significant at $p < 0.05$ (r = 0.27, -0.07, 0.18 and 0.26, respectively). Likewise, Spearman correlation coefficients were not significant at $p < 0.05$. As was expected based on the above findings, when a backwards stepwise multiple linear regression was run with wetland scores on the first four principal components as the predictor variables and $\log_{10}$ mean Hg concentrations as the dependent variable, none of the predictor variables was retained.

When $\log_{10}$ Hg concentrations were plotted against wetland scores on PC1, it was apparent that one wetland, Arrowhead, was an outlier. To determine what influence this wetland had on the results, Arrowhead was removed from the data matrix and a PCA was again run on a correlation matrix of water chemistry. Total Kjeldahl N was also removed so that the number of variables did not exceed the number of observations. Total Kjeldahl N was chosen since it was

highly correlated with DOC (r = 0.94). Loadings of the water chemistry variables onto each principal component were very similar to that obtained when Arrowhead was included. Likewise, none of the correlations between $\log_{10}$ Hg concentrations and principal components were significant at $p < 0.05$. However, the correlations were strengthened.

The structure of the principal components obtained from the PCA of water chemistry was simplified by performing an orthogonal varimax rotation of the first six components. Data for Arrowhead was not included. Six components were chosen as this was found to result in the most simplified structure. The first six components accounted for 96% of the variation in the data set. Loadings of the water chemical variables on these rotated components are listed in Table 10. When Pearson product-moment correlation coefficients were calculated between Hg concentrations and wetland scores on each of the six rotated principal components, only PC1 was significantly correlated with Hg concentrations at $p < 0.05$ (Table 10). Aluminum, colour and DOC had a strong positive influence on the positioning of wetlands along component one. In contrast, sulphate had a somewhat weaker negative influence. Backwards stepwise multiple linear regression, performed with wetland scores on each of the six rotated principal components as the predictor variables and $\log_{10}$ mean Hg concentrations as the dependent variable, retained only PC1. Principal component one explained 40% of the variance in the among wetland nymph Hg concentrations (Table 11). The positive relationship between Hg concentrations in *Cordulia* and wetland scores on rotated PC1 is displayed in Fig. 5.

To further explore the 14 wetland data set, linear regressions were performed between $\log_{10}$ least squares mean Hg concentrations and each variable with an absolute loading greater than 0.5 on rotated PC1 (Table 12). Only square root Al and colour were found to be significant predictors of nymph Hg concentrations at $p < 0.05$. Aluminum explained 38% of the among wetland variation in Hg concentrations, approximately the same amount of variance accounted for by rotated PC1 (40%). Colour explained 34%. Since Al and colour were highly correlated (r = 0.836), it is unclear which, if any, of the two variables are causal factors affecting dragonfly nymph Hg concentrations. The positive relationship between *Cordulia* Hg concentrations and wetland pool water Al concentrations is depicted in Fig. 6.

To be better able to compare the sediment chemistry results with those obtained for water chemistry, Arrowhead was removed from the sediment chemistry data set and $\log_{10}$ Hg concentrations were regressed against Arcsine sediment total S. Sediment total S was able to explain slightly more among wetland variation in nymph Hg concentrations ($r^2 = 0.68$, $p = 0.0003$).

Interestingly, when Arrowhead was removed and Hg concentrations were regressed against %LOI, %LOI was no longer a significant predictor of Hg ($p = 0.08$, $r^2 = 0.24$).

It was suspected that water chemistry may not be directly influencing Hg bioavailability in the wetlands. In fact, it was determined that rotated water chemistry PC1 was significantly negatively correlated with rotated sediment chemistry PC1 (score for Arrowhead removed) ($r^2 = 0.50$; $p = 0.005$). When sediment total S for all wetlands, except Arrowhead, was regressed against scores for each wetland on the rotated water chemistry PC1, PC1 was able to significantly explain 50% of the variation in sediment total S ($p = 0.005$). The negative relationship between sediment total S and rotated PC1 is given in Fig. 7. The significant positive correlation observed between nymph Hg concentrations and wetland scores on rotated water chemistry PC1 appears to have been the result of a confounded relationship between water chemistry variables loading highly onto PC1 and sediment total S.

As a final analysis of the data, a PCA was performed on a correlation matrix of a selected set of water and sediment chemistry variables for 14 wetlands (data for Arrowhead was excluded). The variables included were: DOC, colour, Al, SO4 and Fe (chemical parameters with high loadings on rotated water chemistry PC1), and %LOI and sediment total S. Only the first two components had eigenvalues greater than one (4.27 and 1.30), accounting for 80% of the total variance in the data set. Each of the seven variables included in the correlation matrix had a loading onto PC1 with an absolute value greater than 0.5. Total sediment S and water Fe were the only variables with absolute loadings greater than 0.5 onto PC2. Backwards stepwise multiple linear regression, performed with wetland scores on each of the seven principal components as the predictor variables and log_{10} mean Hg concentrations as the dependent variable, retained two predictor variables (PC1 and PC2). Together these two components were able to account for 61% of the among wetland variance in nymph Hg concentrations ($p = 0.006$). Therefore, the inclusion of the water chemistry variables did not explain any additional amount of the among wetland variance in nymph Hg concentrations than could be accounted for by sediment chemistry alone.

Discussion

Mean total Hg concentrations measured in *Cordulia sp.* (Family: Corduliidae) sampled from 15 wetlands ranged from 0.04 to 0.40 $\mu g.g^{-1}$ dry mass. These levels are comparable to

those reported for Anisoptera inhabiting remote lakes. For five species of Anisoptera collected from a lake in the Haliburton region of south-central Ontario, measured Hg concentrations ranged from about 0.05 to 0.45 $\mu g.g^{-1}$ dry mass (Allan Wong, University of Toronto, pers. comm.). Parkman and Meili (1993) determined total Hg levels in a number of invertebrate species collected from eight remote Swedish forest lakes. Mercury concentrations ranged from 0.09 to 0.59 $\mu g.g^{-1}$ dry mass for Anisoptera nymphs from the families Libellulidae and Aeschnidae. As in the present study, Parkman and Meili (1993) did not find a significant correlation between Hg concentration and nymph dry mass. The dragonfly nymphs analyzed by Parkman and Meili (1993) expanded a much broader weight range (0.003 to 0.28 g) than the nymphs analyzed in this study, which typically weighed between 0.02 and 0.07 g.

When a PCA was performed on a correlation matrix of the wetland water chemistry, the rotated principal component with high positive loadings of Al, colour and DOC was significantly positively correlated with nymph Hg concentrations, accounting for 40% of the among wetland variation in Hg concentrations. Significant positive correlations between water chemical variables representative of dystrophy, and Hg concentrations in fish and crayfish have been documented (McMurtry et al. 1989; Swain and Helwig 1989; Wren et al. 1991; Fjeld and Rognerud 1993; Headon 1994). Miskimmin et al. (1992) demonstrated *decreased* net CH_3Hg production in lake water with increased DOC levels. This lead them to hypothesize that elevated fish Hg concentrations observed in highly coloured lakes resulted from allochthonous inputs of CH_3Hg associated with organics rather than direct CH_3Hg formation within the lake. Detailed monitoring of Hg levels in catchment run-off has suggested that both total Hg (Mierle and Ingram 1991) and CH_3Hg (Lee and Hultberg 1990) are transported from catchments in association with organic substances.

For the present investigation, colour appeared to be better descriptor of Hg concentrations in dragonfly nymphs than DOC. Mierle and Ingram (1991) measured DOC, colour and Hg in headwater streams located on the Precambrian Shield and concluded that the seasonal pattern noted for Hg in stream water was more closely related to colour than DOC. It was apparent from the observed difference in seasonal patterns of DOC and colour that these two parameters did not measure equivalent attributes of the stream water (Mierle and Ingram 1991). Dissolved organic carbon measures humic and fulvic matter (humic material), as well as non-coloured organic substances, whereas colour measures coloured substances, primarily humic material (Mierle and Ingram 1991). The ability of humic material to complex with Hg has been established

in laboratory studies (Lodenius et al. 1983; Thanabalasingam and Pickering 1985).

Positive correlations have been observed between water column Al concentrations and Hg concentrations in fish (McMurtry et al. 1989; Swain and Helwig 1989; Grieb et al. 1990; Wren et al. 1991) and crayfish (Headon 1994). However, this does not imply that Al is a causal factor of increased Hg concentrations in biota. Since acidification leads to elevated concentrations of aqueous Al in surface waters (reviewed in Nelson and Campbell 1991), pH rather than Al may be the parameter influencing Hg concentrations in biota. However, in the present study, the rotated principal component with high loadings of variables representative of water acidity was not significantly correlated with Hg levels ($r^2 = 0.002$, $p = 0.88$). Wetland pool water pH ranged from 5.2 to 7.8. Parkman and Meili (1993) also found that Hg concentrations in dragonfly nymphs collected from remote Swedish lakes were not significantly correlated with pH. However, highest Hg concentrations were obtained for Anisoptera from the most acidic coloured lakes. For this investigation, it was surprising that a significant correlation between Hg concentrations and acidity was not evident, given a reported occurrence of increasing net CH_3Hg production with decreasing water pH in both lake water (Xun et al. 1987; Miskimmin et al. 1992) and at the sediment-water interface (Xun et al. 1987), and the relatively large number of studies which have reported a strong negative correlation between pH and Hg concentrations in fish (e.g. Cope et al. 1990; Suns and Hitchin 1990; Wren et al. 1991). Therefore, it appears that chemical parameters other than pH are important in determining Hg bioavailability in wetland environments.

Water column Al concentrations may be indicative of watershed processes. The significant positive correlation observed between Al and nymph Hg concentrations ($r^2 = 0.38$, $p = 0.019$) may be incidental to the highly significant positive correlation observed between Al and colour ($r^2 = 0.84$). For five lakes located near Sudbury, Ontario, catchment runoff was determined to be the major contributor of Al, accounting for 82-100% of the total Al input (Dillon et al. 1988). If the catchments were a primary source of Al to the study wetlands, then processes that affected the loading of organics from the catchments into the wetlands could have also affected Al loading.

Wetland sediment chemistry accounted for more variation in the among wetland nymph Hg concentrations than water chemistry. When a PCA analysis was conducted on a correlation matrix of sediment chemistry, the principal component with high loadings of %LOI and total S was found to be a significant predictor of Hg concentrations, accounting for 58% of the among wetland variation in Hg concentrations. A number of studies have demonstrated that sediment organic content modifies Hg bioavailability to biota (Breteler et al. 1981; Langston 1982; Di Giulio and

Ryan 1987; Jackson 1988; Parkman and Meili 1993). Based on a detailed survey of Hg distribution in estuarine sediments and Hg concentrations in two deposit-feeding marine bivalves, Langston (1982) concluded that Hg concentrations in these bivalves were best related to total particulate Hg concentrations normalized to percent organic matter. However, percent organic matter was less than 10% for the above estuarine sediments (Langston 1982), which was much lower than values obtained for the wetlands in this study. Breteler et al. (1981) conducted a study of Hg bioavailability in salt marshes that were either experimentally contaminated, 'clean', or industrially contaminated. Sediment organic matter content ranged from 10 to 50%. As the organic content of the salt marshes decreased, Hg concentrations in mussels (*Modiolus demissus*) and fiddler crabs (*Uca sp.*) increased (Breteler et al. 1981).

With respect to Anisoptera nymphs, Parkman and Meili (1993) noted a positive trend between Hg concentrations in these invertebrates and Hg concentrations in lake sediments normalized to carbon content. However, such a relationship was not observed for chironomids in the field (Parkman and Meili 1993). In fact, Hg associated with the operationally defined humic fraction of the sediment (NaOH-extractable) was found to be available to chironomids in laboratory studies (Parkman 1993). The availability of Hg associated with humics has not been determined for Anisoptera. Considering that 23 to 66% of the total Hg in the upper 2 cm of lake sediment samples was found to be associated with the operationally defined humic fraction of the sediment (Parkman 1993), the availability of humic bound Hg to predatory macroinvertebrates in wetlands should be ascertained. Percent LOI is likely not a good surrogate for humic content since the quantity of organic material extracted with NaOH represents less than 25% of the total organic material in estuarine and lake sediments (Luoma and Bryan 1981; Parkman 1993). However, it is conceivable that Hg bound to the humic fraction of particulate organic material may only be available to an organism if directly ingested. Different environmental factors could be important in determining Hg levels in detritivores compared with organisms belonging to higher trophic levels, such as Anisoptera. If only a small portion of the whole body Hg burden in chironomids is in the form of CH_3Hg, then little of the accumulated Hg in chironomids would be expected to be assimilated by organisms at higher trophic levels. Unfortunately, information on CH_3Hg concentrations in chironomids could not be located. Nevertheless, it has been shown that less than 2% of the total Hg in sediment is in the form of CH_3Hg (Campbell et al. 1988; Parkman 1993).

For predatory invertebrates inhabiting aquatic systems, factors affecting CH_3Hg loading

to the aquatic system, net CH_3Hg production at the sediment - water interface and in the water column, the availability of inorganic Hg for methylation, and the availability of CH_3Hg for assimilation from food and water, could all be important in determining their Hg burdens. Methylmercury is of great interest to investigators since it is the form of Hg which is known to biomagnify in food chains, resulting in practically all of the Hg in fish to be in the form of CH_3Hg (Grieb et al. 1990; Bloom 1992). The facility with which CH_3Hg appears to bioaccumulate in fish seems to be related first to the relative differences in excretion rate, CH_3Hg is excreted more slowly than inorganic species (Kramer and Neidhart 1975; Ruohtula and Miettinen 1975; Pentreath 1976; Sharp et al. 1977), and secondly to a more efficient assimilation from food and water (Kramer and Neidhart 1975; Pentreath 1976; deFreitas 1977). Likewise, for marine invertebrates (benthic shrimp and mussels), Fowler et al. (1978) observed that they are able to assimilate radiolabelled CH_3Hg from water and food more efficiently than the inorganic form. Subsequent depuration of assimilated ^{203}Hg was slower for organisms exposed to $CH_3^{203}HgCl$ than those exposed to $^{203}HgCl_2$ (Fowler et al. 1979). Recently, the burrowing mayfly (*Hexagenia rigida*) was shown in a laboratory experiment to preferentially accumulate CH_3Hg relative to the inorganic form over nine days of exposure to either radiolabelled sediments or water (Saouter et al. 1993).

When the sediment chemistry results for the present wetland study were analyzed in more detail, total S was concluded to be a much better descriptor of Hg concentrations in nymphs than organic content. Total particulate S was able to significantly account for 61% of the among wetland variation in nymph Hg concentrations. Under anaerobic conditions, Hg^{2+} has a high binding affinity with sulphide (S^{2-}) and the resulting HgS complex has a very low solubility constant (Ks = 10^{-52}). Björnberg et al. (1988) proposed that the activity of Hg^{2+} in natural waters is regulated by S^{2-}, which in turn will be strongly affected by redox conditions (pE) and pH. An increase in pE will result in a lowering of the S^{2-} activity with a subsequent increase in Hg^{2+} levels (Björnberg et al. 1988). In a laboratory study, Parkman (1993) observed that altering the pE of the interstitial water affected Hg partitioning in the sediment. When the redox potential was increased, there was an increase in the proportion of Hg associated with the NaOH extractable fraction, along with a corresponding increase in SO_4^{2-} in the interstitial water. Parkman (1993) hypothesized that aeration of the experimental sediments led to oxidation of the sulphides with a subsequent release of associated Hg. The released Hg then became bound to the NaOH extractable fraction which was available to detritivorous chironomids (Parkman 1993).

With a decrease in pH, H_2S formation will increase, as a result of the protonization of S^{2-},

and be volatized from the water column (Björnberg et al. 1988). With less S^{2-} available to bind with Hg^{2+}, more Hg^{2+} should be available for uptake or methylation. As mentioned above, in the present wetland study, the rotated principal component with high loadings of variables representative of water acidity was not significantly correlated with Hg levels in Anisoptera. The S^{2-} levels in the wetland sediments may have been sufficient to obscure any measurable pH effect.

Based on the results of a detailed study dealing with the effects of reservoir impoundment on CH_3Hg production in northern Manitoba, Jackson (1988) concluded that both the availability of sediment associated inorganic Hg and the methylating capability of the microbes in the aquatic environment regulates the production of CH_3Hg. In the laboratory, Jackson (1988) observed that S^{2-} inhibited the methylation of inorganic Hg. It was hypothesized that sulphide competed with methylators for Hg^{2+} (Jackson 1988). Jackson (1988) concluded from the study results that sulphide poor anaerobic environments which have high levels of organic nutrients (used as a growth substrate for methylating bacteria) are very favourable for the methylation of Hg. Unfortunately, only sediment total S and not S^{2-} was determined in this study. Assuming that the total S measurements were a fairly good surrogate for S^{2-} levels in these wetland sediments, the negative correlation observed between sediment total S and Hg concentrations in nymphs may have been the result of decreased availability of Hg^{2+} for methylation and/or decreased availability of CH_3Hg formed at the sediment-water interface. Methylmercury can also complex with S^{2-} (Dyrssen and Wedborg 1991).

Further statistical analysis of the wetland data revealed that the significant positive correlation observed between nymph Hg concentrations and wetland scores on rotated water chemistry PC1 was likely the result of a confounded relationship between water chemistry variables representative of dystrophy and sediment total S. However, the observed negative correlation between dystrophy and sediment total S may not have been the result of a causal relationship between these parameters, rather it may have been an artifact of the physical attributes of the wetlands. The four wetlands that are located in the upper left hand area of Fig. 7 (Wolfhowl, Bore, Upper Dawson and Ideal) were originally classified as bogs by Bendell Young et al. (1992). This classification was based on vegetative characteristics, presence or absence of obvious inflows, and water chemistry (alkalinity and pH) (Bendell Young et al. 1992). However, according to the classification system of National Wetlands Working Group (1988), a bog should have a pH < 4.8. The pool water pH's for these four wetlands ranged from 5.2 to 6.1, somewhat

higher than what would be expected for a true bog. Nevertheless, when the four wetlands in question were sampled, there were no obvious inflows of water (a characteristic of bogs). Therefore, these wetlands should have received very little in the way of hydrologic inputs from the watershed, and hence relatively low levels of allochthonous organic matter. This would explain the positioning of these wetlands along the rotated principal component representative of water dystrophy. In addition to these four wetlands, Ike and Towhee also did not have obvious inflows. The remaining nine wetlands all had visible inflows of water, which would explain their relatively high levels of coloured substances in their pool water.

It is not clear why the degree to which a wetland was connected by hydrologic ties to its watershed was related to the quantity of total sediment S. The six wetlands with no obvious inflows of water were vegetatively dominated by *Sphagnum*. The other nine wetlands were vegetatively dominated by grasses and sedges. Perhaps the combination of the quantity of organic material (%LOI), which would explain its weak correlation with nymph Hg concentrations, and the type or characteristics of the organic material (i.e. woody, herbaceous or sphagnum derived) is related to the sediment total S content in wetland environments. The relevant combination of factors that are correlated with high total sediment S levels may tend to be greater in wetlands with little or no hydrologic inputs.

It is somewhat disconcerting that water chemistry was only significantly correlated with Anisoptera nymph Hg levels when one of the 15 wetlands (Arrowhead) was dropped from the analysis. Why this wetland was an outlier is uncertain. It may be due to the fact that the nymphs collected and analyzed for Hg from Arrowhead were all very large. The five individuals had dry masses ranging from 0.075 to 0.087 g; whereas, nymphs sampled from each of the other study wetlands had masses spanning a lower and broader range (usually within 0.02 to 0.07 g). However, based on a plot of the rotated PC1 obtained for sediment chemistry versus rotated PC1 obtained for water chemistry (analyzed data included Arrowhead), it was apparent that the reason why Arrowhead was an apparent outlier was not due to the Hg levels in the nymphs but the relationship between its water and sediment chemistry. Arrowhead had relatively lower levels of coloured substances in its water column than would have been expected based on its sediment chemistry (Fig. 8). If water chemistry does not have a direct influence on Hg bioavailability in wetlands, as hypothesized above, then this finding would explain why the principal component with high loadings of water variables representative of dystrophy was only correlated with nymphs Hg concentrations when Arrowhead was removed from the correlation matrix of water chemistry.

Conclusion

For the wetland systems investigated in this study, sediment total S was able to explain the greatest amount of the among wetland variation in Hg concentrations in Anisoptera nymphs. With increasing concentrations of total S there was a decrease in nymph Hg concentrations. Water chemistry appears not to have directly affected Hg bioavailability. However, factors such as pH, alkalinity and DOC could play a more significant role in determining Hg bioavailability in wetlands which contain low levels of total S in their sediment. Assuming that most of the measured total S was in the form of S^{2-}, the negative correlation observed between total S and Hg levels may have been the result of decreased availability of Hg^{2+} for methylation and/or decreased bioavailability of CH_3Hg formed at the sediment-water interface. However, a statistically significant relationship between an environmental parameter and Hg concentrations in biota does not imply a causal relationship. A series of manipulative experiments will have to be run in the future to determine cause and effect relationships and underlying mechanisms.

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TABLE 1. Arithmetic mean Hg concentrations and dry masses of *Cordulia sp.* collected from the 15 study wetlands. Wetland abbreviations are in parentheses.

Wetland	n	Hg (µg.g^{-1} dry mass)		Dry mass (g)	
		Mean	SE	Mean	SE
Arrowhead (AH)	5	0.402	0.046	0.080	0.002
Deer (DE)	6	0.279	0.016	0.040	0.006
Illegal (IL)	7	0.166	0.025	0.039	0.007
Simp (SI)	7	0.142	0.019	0.059	0.006
N	5	0.139	0.020	0.043	0.009
Ideal (ID)	15	0.134	0.019	0.051	0.005
Number 7 (7)	7	0.110	0.009	0.057	0.007
Harp (HP)	9	0.107	0.013	0.045	0.003
Towhee (TO)	8	0.073	0.008	0.043	0.005
Upper Dawson (UD)	15	0.073	0.009	0.049	0.003
H	5	0.069	0.010	0.055	0.004
Buck (BU)	4	0.054	0.009	0.042	0.009
Bore (BO)	13	0.053	0.006	0.053	0.003
Ike (IK)	18	0.046	0.004	0.049	0.004
Wolfhowl (WH)	6	0.041	0.004	0.056	0.004

TABLE 2. Means and ranges of water and sediment chemical parameters included in the PCA matrices for the 15 wetlands from which *Cordulia sp.* were analyzed for total Hg.

Chemical parameter	Mean	Range
pH	6.12	5.20 - 7.75
Alkalinity (μeq.L^{-1})[1]	176.2	4.8 - 920.4
Conductivity (μS.cm^{-1})	32.2	13.2 - 107.0
DIC (mg.L^{-1})[2]	3.46	0.97 - 10.70
DOC (mg.L^{-1})[3]	10.5	5.5 - 16.0
Colour (true colour units)	105.1	27.0 - 233.0
Total Fe (μg.L^{-1})	1047	48 - 3570
Total Mn (μg.L^{-1})	61	3 - 428
Total Al (μg.L^{-1})	131	10 - 461
SO$_4$ (mg.L^{-1})	3.63	0.75 - 5.60
Nitrates (μg.L^{-1})	7	1 - 26
Total Kjeldahl N (mg.L^{-1})	0.697	0.380 - 1.180
Silicates (mg.L^{-1})	0.76	0.04 - 2.86
F (μg.L^{-1})	54.7	31.6 - 71.1
Dissolved oxygen (mg.L^{-1})	6.2	1.6 - 10.3
Sediment loss on ignition (LOI) (%)	63.5	19.0 - 85.0
Fe in sediment (mg.g^{-1} dry mass)[4]	6.7	2.5 - 17.0
Mn in sediment (μg.g^{-1} dry mass)[4]	119	49 - 208
Total S in sediment (%)	0.591	0.268 - 1.098

[1]Total inflection point alkalinity.
[2]Dissolved inorganic carbon.
[3]Dissolved organic carbon.
[4]Non-residual; strong acid leach.

TABLE 3. Pearson correlation coefficients between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and four sediment chemical parameters. Spearman rank correlation coefficients for least squares geometric mean Hg concentrations and raw chemical parameters are provided for comparison.

Parameter	n	Transformation	Pearson (r)	Spearman (r_s)
LOI	15	none	-0.602*	-0.324
Fe	15	$\log_{10}$	-0.037	-0.046
Mn	15	$\log_{10}$	0.106	0.079
S	15	Arcsine	-0.781*	-0.782*

*$p < 0.05$; to maintain an overall significance of $p < 0.05$, Bonferroni adjusted correlation coefficients are $|r| > 0.641$ and $|r_s| > 0.654$.

TABLE 4. Loadings of sediment parameters onto the first three principal components produced from a PCA analysis of a correlation matrix of sediment chemistry (n = 15). Loadings with an absolute value greater than 0.5 have been underlined.

Parameter	PC1	PC2	PC3
LOI	-0.956	0.211	-0.034
Arcsine S	-0.476	0.843	0.176
Log Fe	0.649	0.560	0.495
Log Mn	0.392	0.610	-0.687
% Total variance explained	42.9	36.0	18.7

TABLE 5. Results of a backwards stepwise multiple linear regression of least squares $\log_{10}$ mean nymph Hg concentrations on principal components. Principal components were produced from a PCA analysis of a correlation matrix of sediment chemistry (n = 15).

Variable	Coefficient	SE	t	p
Intercept	-1.020	0.050	-20.61	0.0000
PC1	0.164	0.051	3.19	0.0086
PC2	-0.150	0.051	-2.92	0.0139
PC3	-0.081	0.051	-1.59	0.1411

$r^2 = 0.659$; $p = 0.0064$

TABLE 6. Loadings of sediment parameters onto two rotated principal components produced from a PCA analysis of the correlation matrix of sediment chemistry for the 15 study wetlands. Pearson correlation coefficients (r) between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and wetland scores on each of the rotated components are also provided. Loadings with an absolute value greater than 0.5 have been underlined.

Parameter	PC1	PC2
LOI	<u>0.860</u>	-0.468
Arcsine S	<u>0.912</u>	0.324
Log Fe	-0.122	<u>0.849</u>
Log Mn	0.105	<u>0.718</u>
% Total variance explained	39.9	39.0
r	-0.762*	-0.019

*p = 0.001

TABLE 7. Results of a backwards stepwise multiple linear regression of least squares $\log_{10}$ mean nymph Hg concentrations on rotated principal components for 15 wetlands. Principal components were produced from a PCA analysis of a correlation matrix of sediment chemistry.

Variable	Coefficient	SE	t	p
Intercept	-1.020	0.051	-20.20	0.0000
PC1	-0.222	0.052	-4.24	0.0010

$r^2 = 0.580$; $p = 0.001$

TABLE 8. Pearson product-moment correlation coefficients between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and 15 water chemical parameters (n = 15). Spearman rank correlation coefficients for least squares geometric mean Hg concentrations and raw chemical parameters are provided for comparison.

Parameter	Transformation	Pearson (r)	Spearman (r_s)
pH	none	-0.136	-0.050
Alkalinity	Log_{10}	-0.025	-0.018
Conductivity	inverse	0.016	0.029
DIC	Log_{10}	-0.050	-0.075
DOC	Log_{10}	0.185	0.295
Colour	none	0.344	0.311
Total Fe	Log_{10}	0.220	0.257
Total Mn	Log_{10}	0.269	0.375
Total Al	square root	0.459	0.350
SO_4	none	-0.074	-0.131
Nitrates	Log_{10}	0.304	0.290
Total Kjeldahl N	Log_{10}	0.082	0.179
Silicates	Log_{10}	0.177	0.159
F	none	0.150	0.232
Dissolved oxygen	none	-0.041	-0.025

TABLE 9. Loadings of water chemical parameters onto the first four principal components produced from the PCA analysis of the correlation matrix of water chemistry for the 15 study wetlands. Loadings with an absolute value greater than 0.5 have been underlined.

Parameter	PC1	PC2	PC3	PC4
Colour	0.934	0.261	0.062	0.103
Log Fe	0.871	-0.116	0.292	-0.050
Log Mn	0.859	-0.112	0.010	-0.302
Square root Al	0.847	-0.154	0.106	0.335
SO$_4$	-0.803	0.178	0.403	0.011
Log Si	0.769	0.122	0.352	-0.067
Dissolved oxygen	-0.682	0.272	0.104	0.572
Log DOC	0.677	0.539	-0.238	0.277
Log TKN	0.663	0.519	-0.179	0.185
Square root alkalinity	-0.117	0.978	0.020	-0.063
Log conductivity	-0.169	0.955	0.078	-0.123
Log DIC	0.226	0.885	-0.092	-0.338
pH	-0.355	-0.879	0.165	0.073
Log nitrates	0.161	-0.036	0.904	0.338
F	0.126	0.025	-0.463	0.656
% Total variance explained	39.7	28.2	10.4	8.9

TABLE 10. Loadings of water chemical parameters onto six rotated principal components produced from a PCA analysis of the correlation matrix of water chemistry for 14 of the 15 study wetlands. Pearson correlation coefficients (r) between least squares $\log_{10}$ mean whole-body nymph Hg concentrations and wetland scores on each of the rotated components are also provided. Loadings with an absolute value greater than 0.5 have been underlined.

Parameter	PC1	PC2	PC3	PC4	PC5	PC6
Log DOC	0.900	0.327	-0.020	0.080	0.160	-0.024
Colour	0.747	0.116	0.185	0.063	0.595	0.110
Square root Al	0.711	-0.279	0.301	0.183	0.417	0.158
SO_4	-0.560	0.308	0.284	-0.049	-0.467	-0.506
Square root alkalinity	0.077	0.984	-0.010	-0.016	-0.124	0.009
Log conductivity	0.016	0.963	0.016	-0.035	-0.070	-0.234
pH	-0.173	0.916	0.166	0.090	-0.275	0.027
Log DIC	0.229	0.895	-0.213	-0.117	0.253	0.045
Log nitrates	0.110	-0.027	0.971	-0.107	0.033	-0.013
F	0.129	-0.058	-0.098	0.975	-0.010	-0.010
Log Mn	0.339	-0.094	-0.042	0.035	0.886	0.125
DO	-0.162	0.257	0.248	0.240	-0.853	0.097
Log Si	0.195	0.152	0.385	0.20	0.837	0.132
Log Fe	0.482	-0.158	0.310	-0.177	0.592	0.459
% Total variance explained	19.4	28.3	11.0	8.3	25.0	4.3
r	0.631*	-0.043	0.196	0.254	0.039	-0.083

$^{*}p < 0.05$; to maintain an overall significance of $p < 0.05$, the Bonferroni adjusted correlation coefficient is $|r| > 0.661$.

TABLE 11. Backwards stepwise multiple linear regression of least squares $\log_{10}$ mean nymph Hg concentrations on rotated principal component scores for 14 of the 15 study wetlands. Principal components were produced from a PCA analysis of a correlation matrix of water chemistry.

Variable	Coefficient	SE	t	p
Intercept	-1.063	0.053	-20.05	0.0000
PC1	0.155	0.055	2.82	0.0155

$r^2 = 0.398; p = 0.0155$

TABLE 12. Linear regressions between $\log_{10}$ least squares mean Hg concentrations in *Cordulia sp.* and water column DOC, colour, aluminum and sulphate for 14 of the 15 study wetlands.

Variable	Slope	Intercept	r^2	p
Square root Al	0.034	-1.425	0.379	0.019
Colour	0.002	-1.292	0.339	0.029
Log DOC	0.775	-1.844	0.274	0.055
SO_4	-0.055	-0.869	0.092	0.293
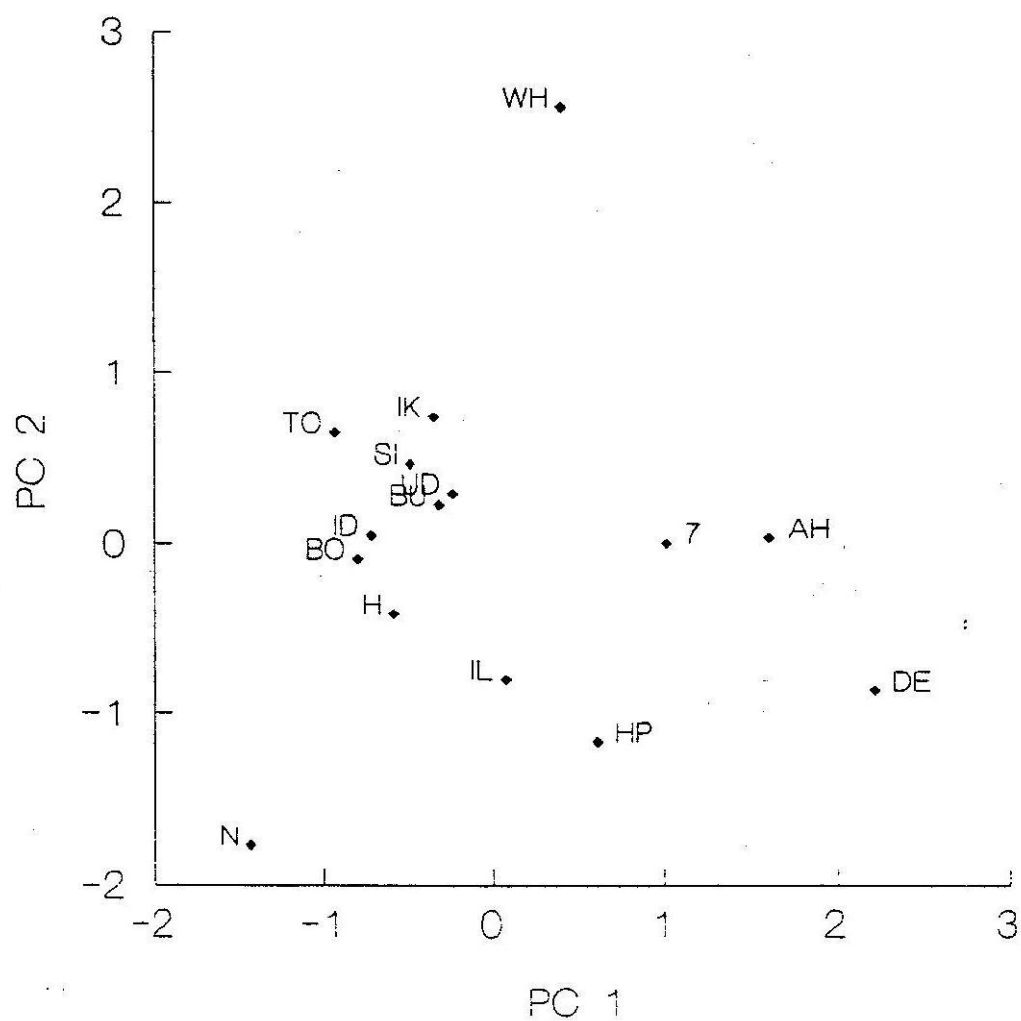
Figure 1. Scores of the 15 study wetlands on principal component two versus wetland scores on principal component one. Principal components were obtained by conducting a PCA on a correlation matrix of sediment chemistry (principal components were *not* rotated). Abbreviations for wetlands are listed in Table 1.
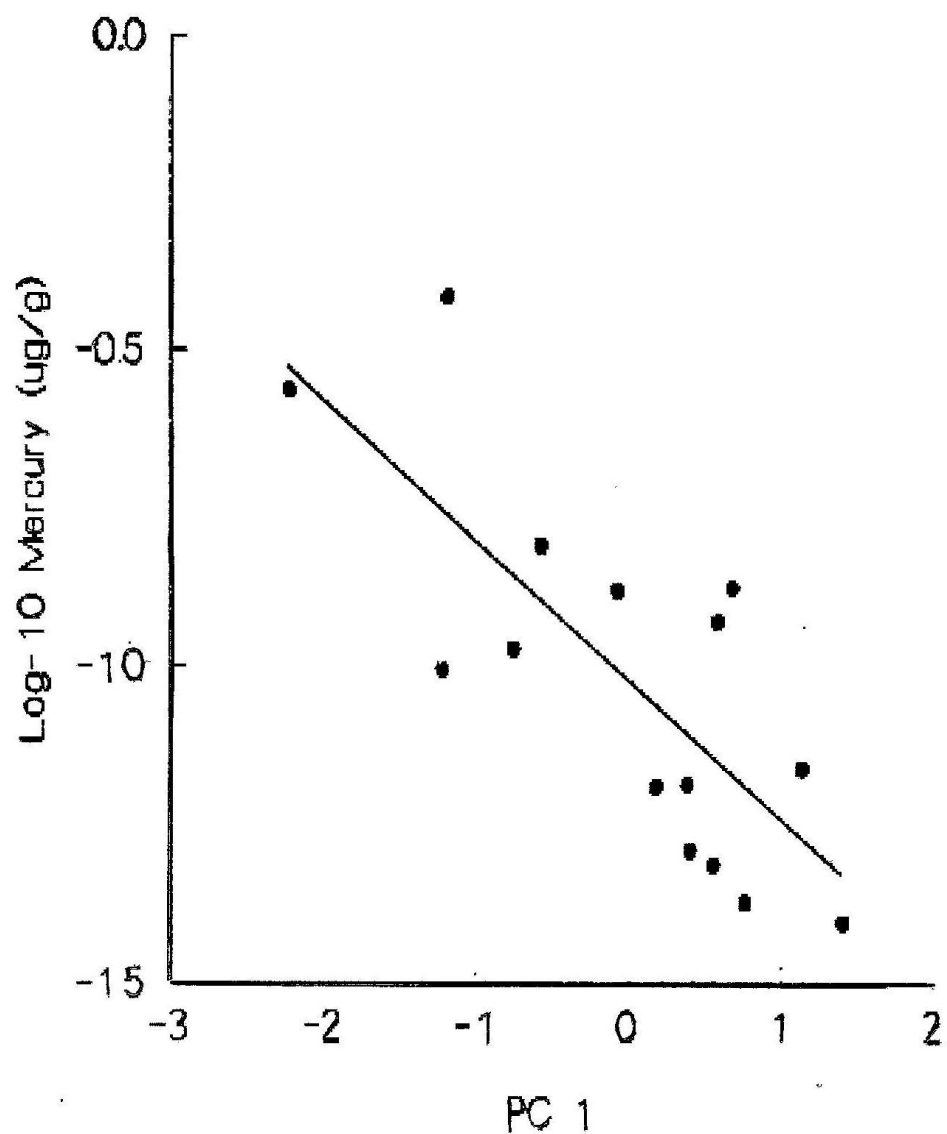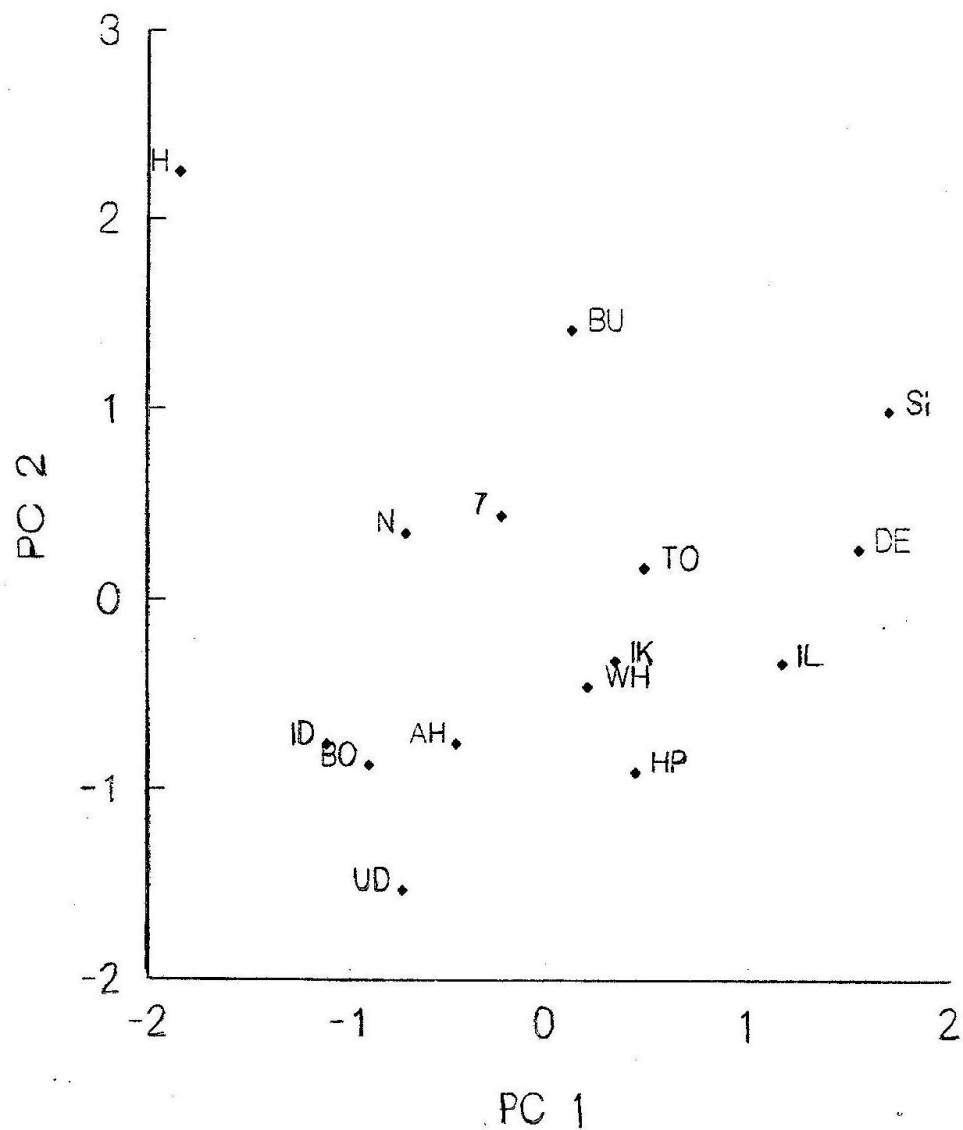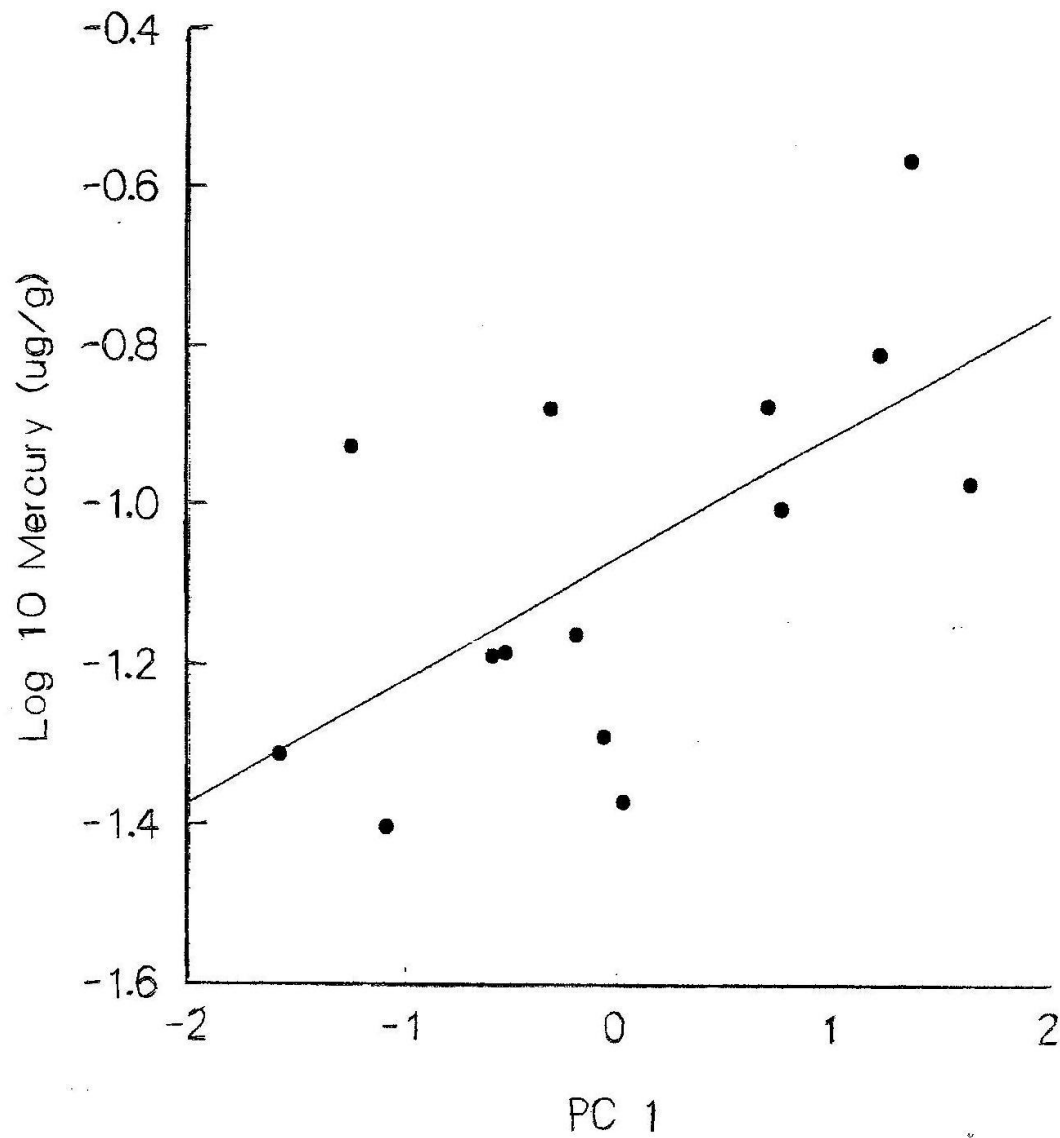
Figure 2. Mercury concentrations in *Cordulia sp.* in relation to wetland scores on rotated principal component one, where:

Log_{10} Hg (µg.g^{-1} dry mass) = -1.020 – 0.222*PC1 (n = 15, r^2 = 0.58, p = 0.001)

Scores were obtained by conducting a PCA on a correlation matrix of sediment chemistry.
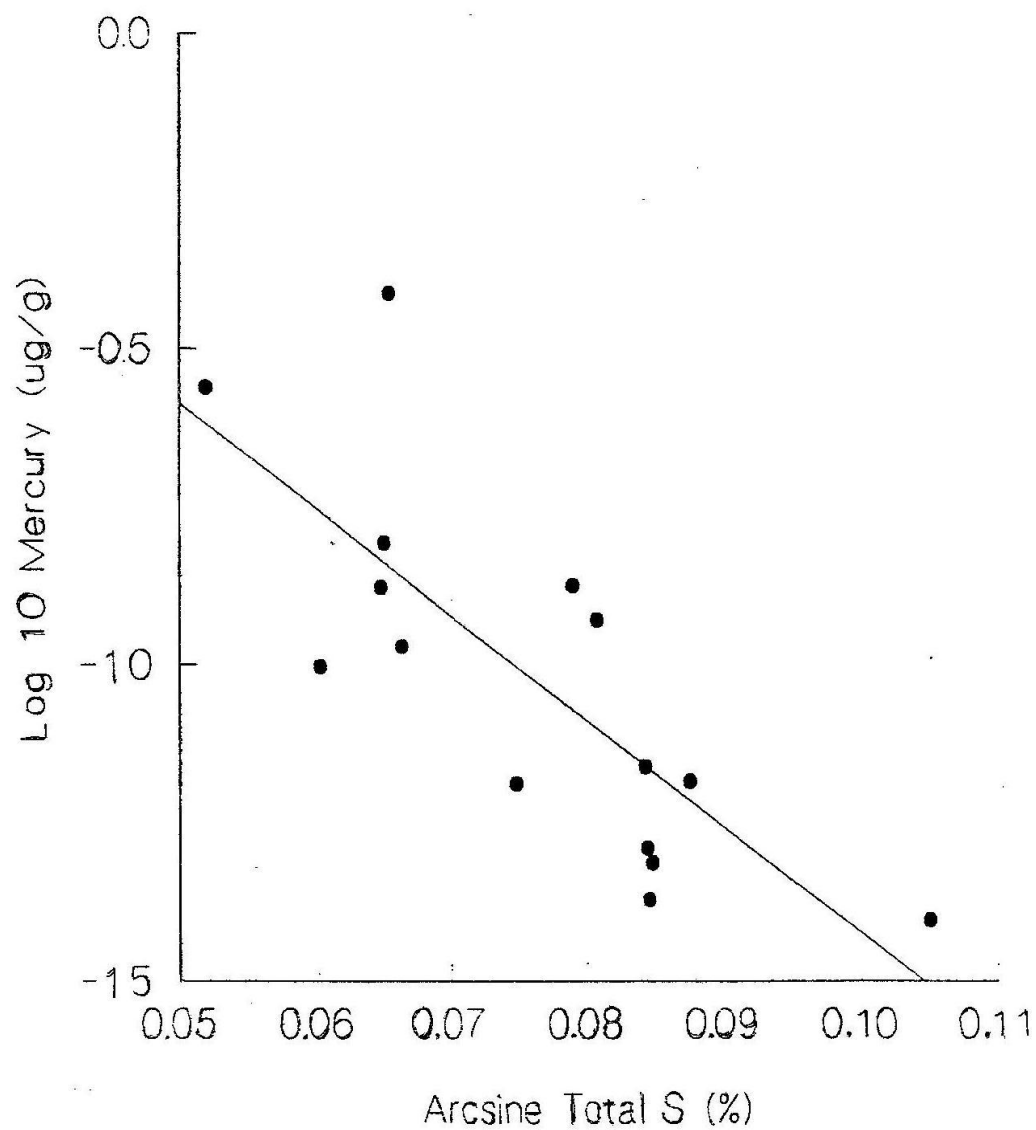
Figure 3. Mercury concentrations in *Cordulia sp.* in relation to total sulphur in sediment of 15 wetlands, where:

Log_{10} Hg ($\mu g.g^{-1}$ dry mass) = 0.249 – 16.729*Arcsine S (%) (r^2 = 0.61, p < 0.001)

Figure 4. Scores of the 15 study wetlands on principal component two versus wetland scores on principal component one. Principal components were obtained by conducting a PCA on a correlation matrix of water chemistry (principal components were *not* rotated). Abbreviations for wetlands are listed in Table 1.

Figure 5. Mercury concentrations in *Cordulia sp.* in relation to wetland scores on rotated principal component one, where:

Log_{10} Hg ($\mu g.g^{-1}$ dry mass) = -1.063 + 0.155*PC1 (n = 14, r^2 = 0.40, p = 0.016)

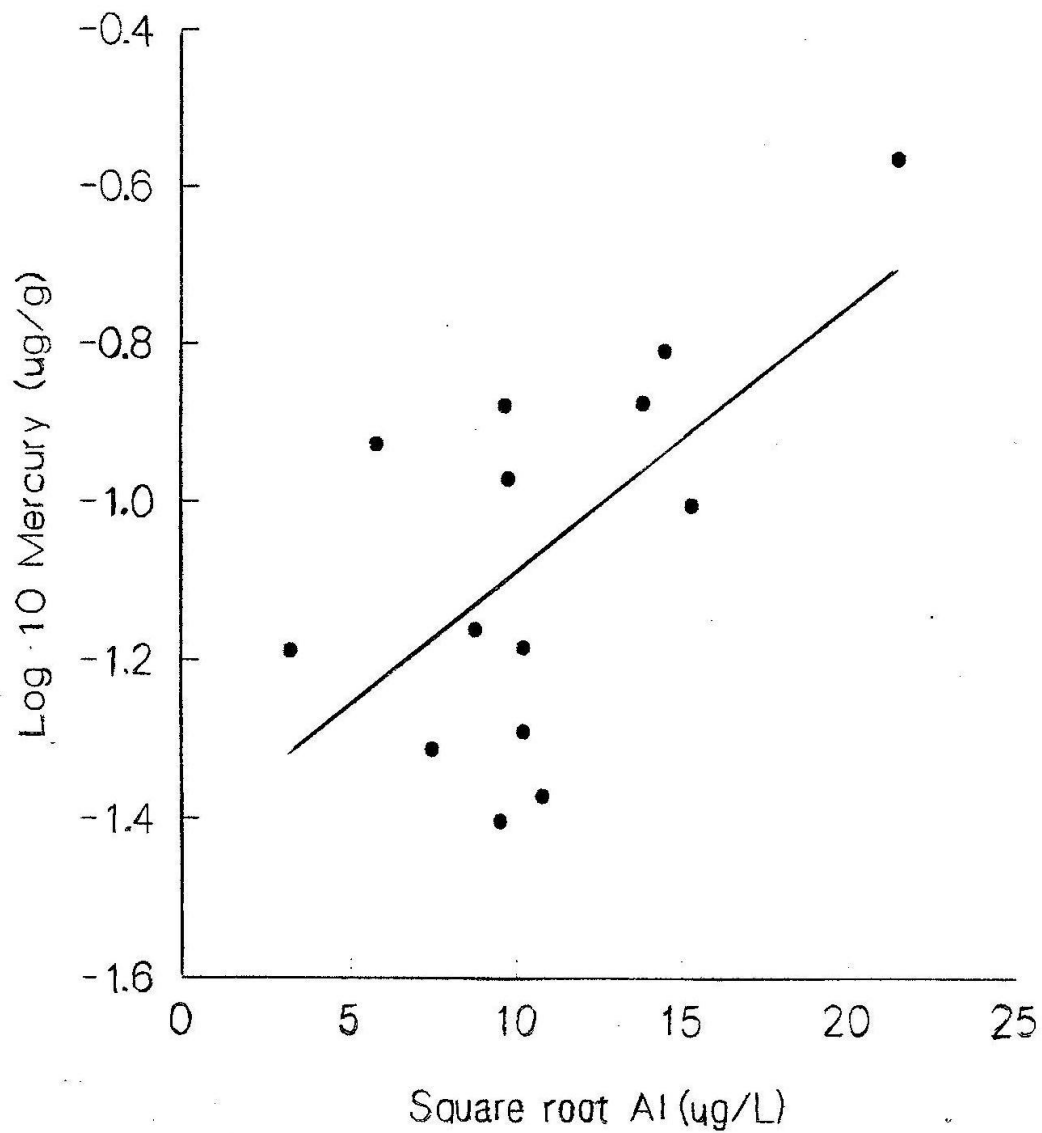Scores were obtained by conducting a PCA on a correlation matrix of wetland water chemistry.

Figure 6. Mercury concentrations in *Cordulia sp.* in relation to wetland pool water Al
concentrations for 14 wetlands, where:

Log_{10} Hg (μg.g^{-1} dry mass) = -1.425 + 0.034*Square root Al (μg.L^{-1}) (r^2 = 0.38, p = 0.019)
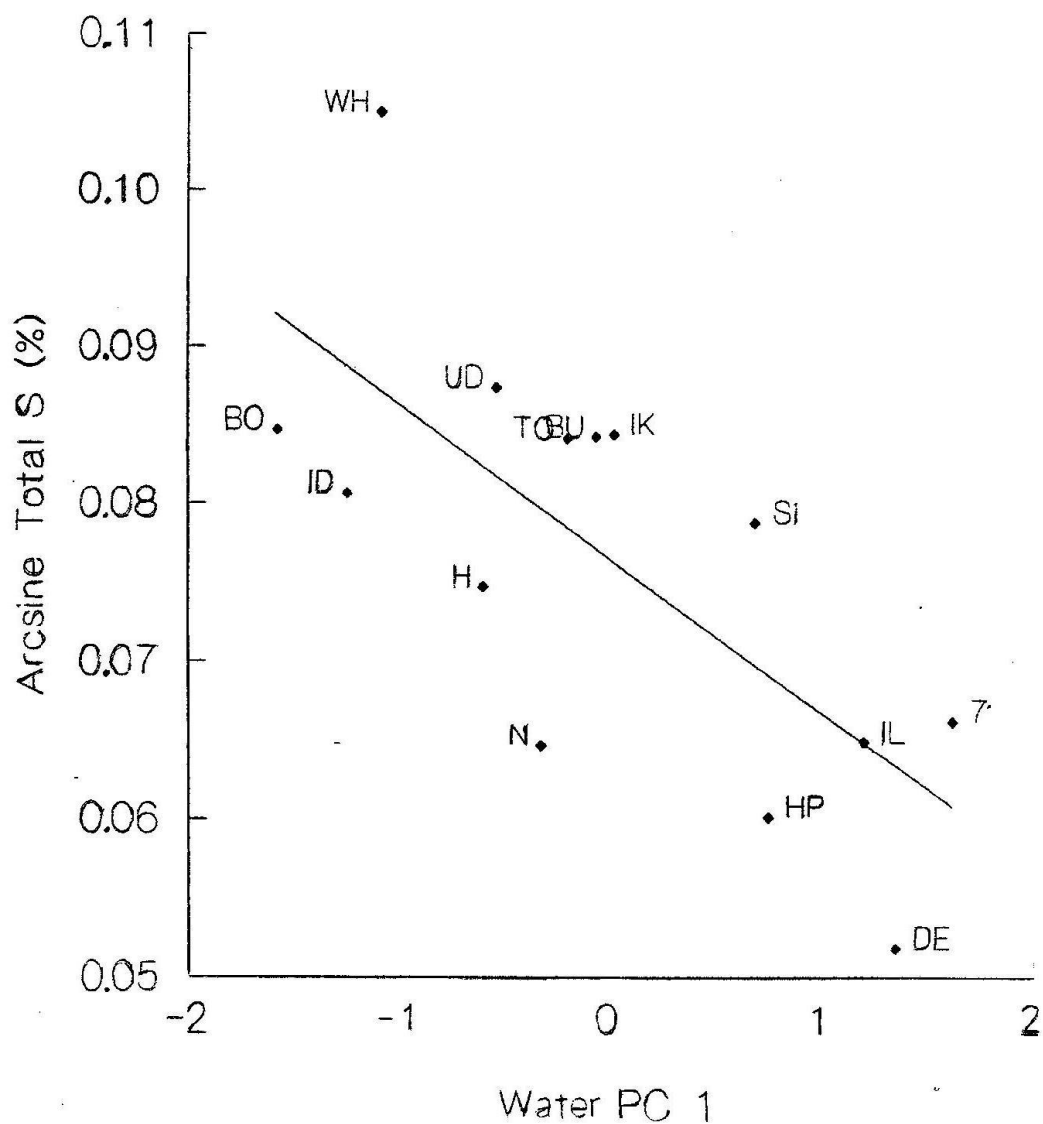
Figure 7. Total S in sediment in relation to wetland scores on rotated principal component one, where:

Arcsine S (%) = 0.077 – 0.010*PC1 (n = 14, r^2 = 0.50, p = 0.005)

Scores were obtained by conducting a PCA on a correlation matrix of wetland water chemistry (n = 14). Abbreviations for wetlands are listed in Table 1.
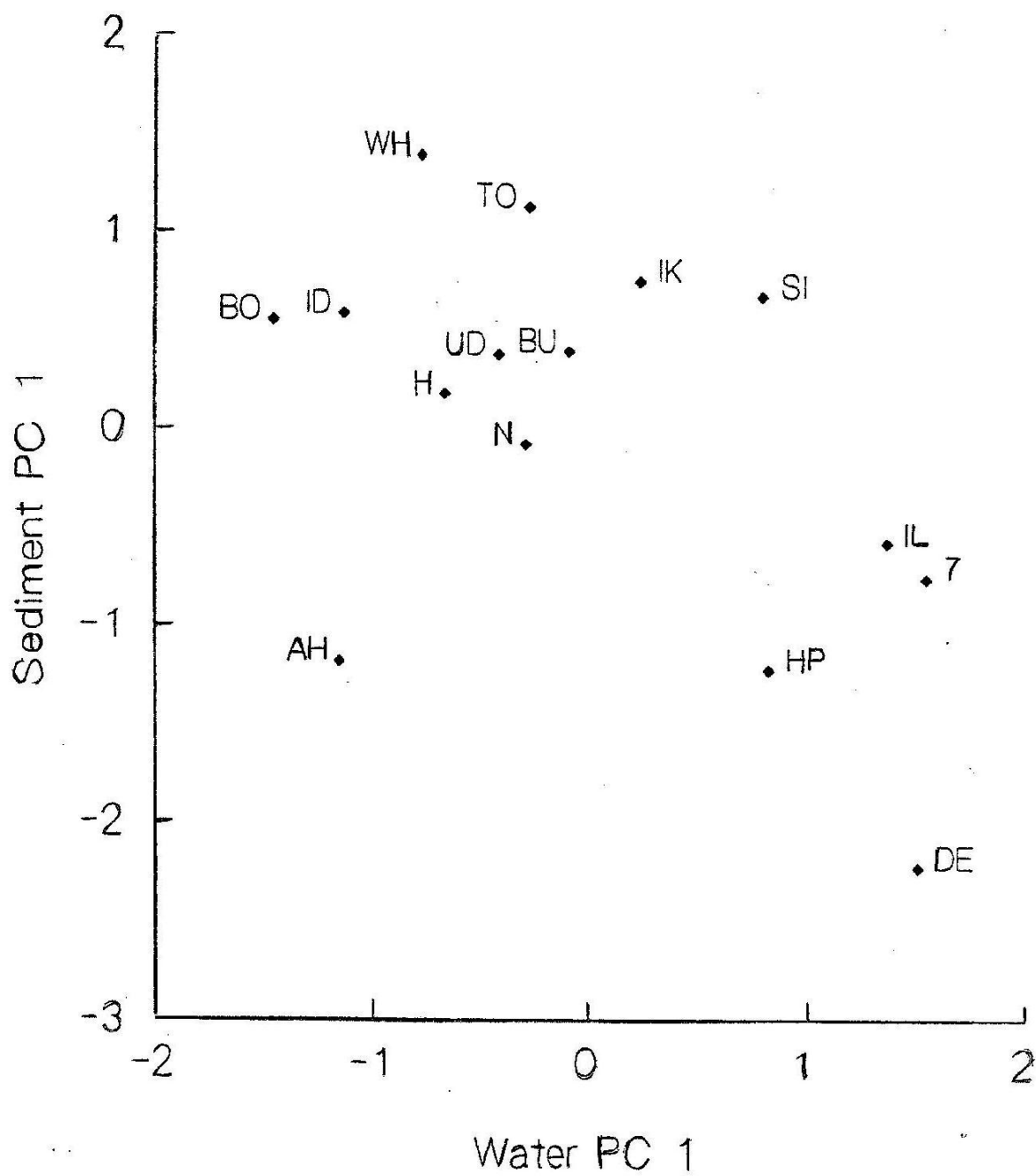
Figure 8. Relationship between rotated PC1 for sediment chemistry (included AH) and rotated PC1 for water chemistry. Abbreviations for wetlands are listed in Table 1.